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Necrotizing enterocolitis: the quest for biomarkers

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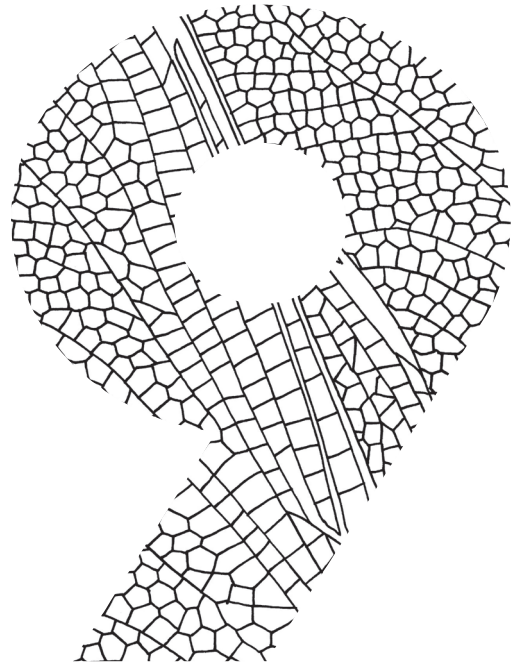
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General discussion

Despite decades of intensive research, our understanding of the pathophysiology of necrotizing enterocolitis remains poor. The common denominator for development of necrotizing enterocolitis in preterm infants seems to be intestinal immaturity. This immaturity is believed to lead to decreased circulatory regulation, diminished intestinal barrier function, altered microbiota and an impaired immune defense.¹⁻⁵ These risk factors can, especially when combined, induce an uncontrolled inflammatory cascade in the intestine that causes the clinical signs regularly seen in necrotizing enterocolitis, such as feeding intolerance, abdominal distension, bloody stools and, if the disease progresses, intestinal perforation, peritonitis and sepsis.

The course of necrotizing enterocolitis can be highly unpredictable, which makes it as yet impossible to foresee which infants are likely to develop a severe disease that necessitates surgery or leads to death.² Surgical procedures in necrotizing enterocolitis can involve exploratory laparotomy with resection of the necrotic bowel followed by the creation of either a primary anastomosis or enterostomy.^{6,7} The creation of an enterostomy seems a safe alternative in neonates with an acute abdomen undergoing surgery. There is, however, little known about the rate and type of complications involved with the creation and closure of an enterostomy.

Due to the fulminant nature of necrotizing enterocolitis, it is unlikely that new treatment strategies will provide any major breakthroughs in reducing necrotizing enterocolitis-associated mortality and morbidity in the near future.⁸ Prevention of necrotizing enterocolitis is likely to yield better results and improve clinical outcomes for preterm infants.^{8,9} To prevent the development of necrotizing enterocolitis, however, it is essential that we accurately predict which neonates are at a high risk of developing the disease.⁹ Understanding the risk factors for necrotizing enterocolitis and identifying indicators that detect these abnormalities early can help us to define markers that aid in the prediction of the disease. As the population of affected patients with necrotizing enterocolitis mainly consists of vulnerable preterm infants for whom invasive techniques (such as frequent blood sampling) are highly undesirable, the ideal marker should be measurable using non-invasive methods.^{10,11}

This thesis is about prediction and early diagnostics of necrotizing enterocolitis, in relation to several non-invasive biomarkers. It also covers various clinical aspects. The **first aim** was to determine noninvasive markers to predict the development of necrotizing enterocolitis in asymptomatic preterm infants. The **second aim** was to determine a possible marker that independently predicts the progression of necrotizing enterocolitis within the first day after diagnosis. Our **third aim**, then, was to assess the rate and type of complications after the creation and closure of an enterostomy in neonates with an acute abdominal emergency in our center. To this end, we formulated the following research questions:

1. What is known in the literature about predicting necrotizing enterocolitis in preterm infants using noninvasive markers? (*Chapter 2*)

2. Can we differentiate high-risk infants who develop necrotizing enterocolitis from those who do not by prospectively measuring urinary intestinal fatty acid-binding protein? (*Chapter 3*)
3. Can serial measurements of fecal calprotectin help us to differentiate high-risk infants who develop necrotizing enterocolitis from those who do not? (*Chapter 4*)
4. Can serial measurements of fecal bile salts aid in identifying which preterm infants have an increased risk for the development of necrotizing enterocolitis? (*Chapter 5*)
5. Can cerebral and intestinal oxygenation differentiate high-risk infants who develop necrotizing enterocolitis from those who do not? (*Chapter 6*)
6. Can conventional laboratory parameters, obtained in an early stage of the disease, be used to predict the progression of necrotizing enterocolitis? (*Chapter 7*)
7. What complications occur after the creation and closure of an enterostomy in neonates with an acute abdominal emergency in our center? Is the creation of an enterostomy a safe alternative or should it preferably be avoided? (*Chapter 8*)

General discussion

Many studies in this thesis are part of the prospective CALIFORNIA study, which aimed to identify several promising non-invasive markers for necrotizing enterocolitis.¹² These studies could also shed light on the pathophysiology of necrotizing enterocolitis, as the markers we tested are all related to different aspects of the pathophysiology of the disease. We analyzed markers of intestinal damage, intestinal inflammation, intestinal perfusion and bile salts metabolism respectively for their role in the identification of infants with an increased risk for the development of necrotizing enterocolitis. Two of the markers (that of intestinal damage and intestinal inflammation) might not be useful (*chapter 3, chapter 4*), whereas the other two (intestinal perfusion and bile salt metabolism) showed more promising results in the prediction of necrotizing enterocolitis (*chapter 5, chapter 6*). Our main findings are presented in Table 1.

We first investigated a marker for enterocyte damage in the prediction of necrotizing enterocolitis. Urinary intestinal fatty acid-binding protein (iFABP_u) is among the most commonly studied potential markers for the prediction of necrotizing enterocolitis (*Chapter 2*).¹³ Given its clear association with intestinal

(enterocyte) damage and reports of its potent ability to predict the development of necrotizing enterocolitis, iFABP could be considered among the most promising biomarkers studied so far.^{13,14} Elevated iFABP_u levels during the first days after birth were suggested to be the initial sign of mucosal damage that predisposes an infant to necrotizing enterocolitis.^{15,16} In previous studies, such high iFABP_u levels immediately after birth have been found in infants who subsequently developed the disease.¹⁵ Furthermore, Gregory *et al.* suggested that iFABP_u is a useful predictor of necrotizing enterocolitis within a week (sens. 60%, spec. 78%) and, even more strongly, within three days (sens. 65%, spec. 84%) preceding the diagnosis.¹⁷ A sensitivity and specificity up to 100% and 96% resp. of iFABP_u at the day prior to the diagnosis of necrotizing enterocolitis were described by Gollin *et al.*¹⁶

We therefore studied iFABP/cr_u ratios prospectively and longitudinally in a large cohort of high-risk infants. We used iFABP to creatinine ratios (iFABP/cr_u) to correct for variations in kidney function and urine concentration. We were surprised that our results contradicted the findings of previous studies. We demonstrated clearly that iFABP/cr_u ratios have no role in the early prediction of imminent necrotizing enterocolitis in preterm infants (*Chapter 3*). That our findings contradict earlier literature might, at least partly, be the result of differences in study design. Other researchers measured iFABP levels up to the time of the confirmed diagnosis of necrotizing enterocolitis.^{16,17} The confirmation of the diagnosis may have been stated at a considerable time after clinical suspicion of necrotizing enterocolitis arose, explaining why iFABP levels were already elevated in these studies. We analyzed the predictive value of iFABP/cr_u ratios much earlier, *until* the first clinical symptoms of the disease, which we defined as the first abdominal x-ray within the episode of suspected necrotizing enterocolitis. In our cohort, iFABP/cr_u did not differ prior to the onset of symptoms between subjects that developed necrotizing enterocolitis and controls. The ratios were, however, significantly higher in subjects with necrotizing enterocolitis immediately after the first clinical suspicion of the disease had arisen. These findings suggest that necrotizing enterocolitis is a disease in which intestinal (enterocyte) damage, detected by an increased level of iFABP, rapidly leads to clinical signs.¹⁴ But detection of intestinal damage prior to the onset of the disease seems therefore not feasible using iFABP measurements in urine. This appears to fit the often very acute clinical course of the disease. The low sensitivity described in literature of iFABP to predict NEC at respectively 7 and 3 days prior to the onset of the disease¹⁷ (60% and 65%, resp.), chimes with this suggestion.

Table 1. Main findings of this thesis

	iFABP/cr _u urine Chapter 3	Calprotectin feces Chapter 4	Bile-salts feces Chapter 5	NIRS	
				Cerebral oxygenation Chapter 6	Intestinal oxygenation Chapter 6
First week after birth	Not significant	Not significant	Not significant (first feces sample after birth)	Infants with r SO ₂ < 70% developed NEC 9 times more often than infants with r SO ₂ ≥ 70% (Day 1, 2 after birth)	Not significant
Prior to clinical suspicion of NEC	Not significant	Not significant	At 5 days prior to NEC, total unconjugated BS were 4-fold higher in NEC than in controls (median 0.65 vs. 0.16 µmol/ g)	Not significant	At 2 days prior to NEC, intFTOE was significantly higher in infants who developed NEC compared with infants who did not (median 0.65 vs. 0.44)
After clinical suspicion of NEC	Levels were significantly higher in infants with NEC compared with controls (median 4.0 vs. 2.4 pg/nmol)	NI	NI	NI	NI

Abbreviations: BS – bile salts; iFABP/cr_u – intestinal fatty acid binding protein to creatinine ratio in urine; intFTOE – intestinal fractional tissue oxygen extraction; NI – not investigated; NEC – necrotizing enterocolitis; r SO₂ – cerebral oxygen saturation;

As necrotizing enterocolitis is an acute inflammatory process, establishing a marker for bowel wall inflammation could theoretically be helpful in its prediction or early detection.¹⁸ Previously, fecal calprotectin had shown high sensitivity and specificity to predict moderate necrotizing enterocolitis in a small number of infants within one day prior to the onset of the disease (*Chapter 2*).¹⁹ Although these results were promising, the effectiveness of using this protein in neonates was still doubted as previously high levels and wide interindividual variations in fecal calprotectin were observed during the first month of life.^{20–24} We therefore measured fecal calprotectin serially during the first five weeks after birth in preterm infants at high-risk for necrotizing enterocolitis. We found no differences between calprotectin concentrations in subjects who developed necrotizing enterocolitis and controls during the week before the first clinical suspicion of the disease. Furthermore, within subjects developing necrotizing enterocolitis we did not detect an intra-individual rise in calprotectin concentrations before clinical symptoms of the disease occurred. Our results confirmed the previous findings of high concentrations and wide interindividual variation in calprotectin.^{20,22,24–26} Additionally, we observed a wide *intra*-individual variation, which further precludes the use of serial calprotectin concentrations in neonatal care in general and, more specifically, in the prediction of necrotizing enterocolitis (*Chapter 4*).

Recently, an aberrant bile salt metabolism has been associated with the development of necrotizing enterocolitis.²⁷ The findings of this thesis suggest that serial measurements of fecal bile salts levels might be useful in the prediction of necrotizing enterocolitis (*Chapter 5*). We measured unconjugated and conjugated fecal bile salts in the first feces sample after birth and in two samples prior to the manifestation of necrotizing enterocolitis in ten infants who developed necrotizing enterocolitis and in twenty matched controls. We observed that total unconjugated bile salt concentrations in preterm infants that develop necrotizing enterocolitis decrease at a slower rate after birth compared to controls. The level of fecal bile salts might be influenced by several factors, such as antibiotic treatment or formula feeding.²⁸ Further studies are needed to determine the factors that affect levels of fecal bile salts in preterm infants, and whether measuring fecal bile salts has sufficient predictive value and clinical applicability for the development of necrotizing enterocolitis. Further research is also necessary to determine whether the levels of bile salts are causally related to the development of necrotizing enterocolitis or whether altered levels of bile salts are just a bystander effect of other factors that are thought to be related to necrotizing enterocolitis e.g. an altered microbiota.²⁹

The last marker that was studied in the CALIFORNIA cohort in this thesis focused on perfusion of both the intestines and the brain (*Chapter 6*). Our findings suggest that measuring cerebral oxygenation might be useful in the prediction of

necrotizing enterocolitis. We found that infants with cerebral oxygen saturation values below 70 percent (below the 25th percentile) in the first two days after birth had a nine-fold higher risk of developing necrotizing enterocolitis than infants with cerebral oxygen saturation above 70 percent (greater than or equal to the 25th percentile). Intestinal oxygenation values obtained in the first two days after birth were not associated with the development of necrotizing enterocolitis later on. At 2 days prior to necrotizing enterocolitis, however, intestinal fractional tissue oxygen extraction (FTOE) was significantly higher in infants who developed necrotizing enterocolitis with infants who did not, indicating that intestinal perfusion may have been impaired in the infants developing necrotizing enterocolitis a few days later. We were, however, only able to measure intestinal oxygenation values in 23% of the infants (*Chapter 6*), particular during the first week after birth. This small sample size may have limited our ability to detect significant differences in intestinal oxygenation values between the two groups. We encountered several practical difficulties in monitoring the infra-umbilical region in the first days after birth. On the very preterm infants and the infants small for their gestational age space was lacking for adequate sensor placement. Furthermore, the presence of an umbilicus venous catheter taped to the abdominal skin impeded sensor placement. These findings indicate that monitoring at the infra-umbilical region is accompanied with practical difficulties in preterm infants in the first days after birth, potentially limiting the usefulness of this measurement procedure in clinical practice.

The clinical presentation of neonates with signs and symptoms that suggest necrotizing enterocolitis can be obscured by similarities to other inflammatory conditions of the newborn.³⁰ Moreover, there are no reliable prognostic signs of progressive necrotizing enterocolitis that prelude disease progression prior to patients reaching an irreversible state requiring surgery or leading to death.³¹ We assessed, in a 20-year cohort of necrotizing enterocolitis infants, the abilities of conventional laboratory parameters obtained at three different time points during the first 24 hours after diagnosis to independently predict the progression of necrotizing enterocolitis in a large population of patients (*Chapter 7*). Metabolic acidosis ($\text{pH} < 7.3$) and thrombocytopenia were found to be the independent predictors for the progression to severe disease. We concluded, therefore, that acidosis and/or thrombocytopenia at onset and during the first 12 to 24 hours after diagnosis of necrotizing enterocolitis should alert clinicians to the considerably increased risk of progressive disease later on.

In conclusion, the findings in this thesis suggest that necrotizing enterocolitis is a disease in which intestinal damage is almost immediately followed by clinical signs. Therefore, markers that detect intestinal damage or intestinal inflammation

might not be useful in the early identification of infants who will develop necrotizing enterocolitis. The high concentrations and wide inter- and intra-individual variation in fecal calprotectin in preterm infants during the first weeks of life preclude their use to differentiate infants who will develop necrotizing enterocolitis from those who will not. Promising results were found for measurements of respectively fecal bile salt levels and cerebral and perhaps intestinal oxygenation. These markers might be useful in the early identification of preterm infants who will develop necrotizing enterocolitis, but further studies are needed to determine their precise predictive value and clinical applicability.

While our findings might not have pointed directly towards a new marker for the prediction of necrotizing enterocolitis, they did offer insights into the pathophysiology of necrotizing enterocolitis. Understanding the factors involved in the pathophysiology of necrotizing enterocolitis can also help us to identify predictive markers in the future.

Pathophysiology

Factors that might be related to the pathophysiology of necrotizing enterocolitis in preterm infants include an impaired intestinal circulation,^{32–34} intestinal cell damage,^{16,17} intestinal inflammation,^{2,5} and intestinal bile salt metabolism.^{27,35} In Figure 1 we present a schematic overview of the insights into the pathophysiology of necrotizing enterocolitis as found in this thesis.

An early postnatal factor that has been associated with the development of necrotizing enterocolitis later in life is the presence of impaired intestinal circulation during the first days of life.^{33,36,37} Indications of impaired splanchnic perfusion were found in the first days after birth in infants who developed necrotizing enterocolitis later on.³³ This impaired perfusion might possibly lead to local, subclinical damage of the mucosal layer that further predisposes the infant to the later development of necrotizing enterocolitis.

In the first two days after birth, we found no differences in intestinal oxygenation values between infants who went on to develop necrotizing enterocolitis and controls (*Chapter 6*). Neither did we observe differences in iFABP/cr_u ratios between the two groups (*Chapter 3*). These results appear to be in line with each other: as no impaired intestinal oxygenation occurred, no ischemic mucosal damage took place. Infants who developed necrotizing enterocolitis, however, had lower cerebral oxygenation values compared to controls. These findings suggest that the systemic circulation is compromised in those infants during the first two days after birth.

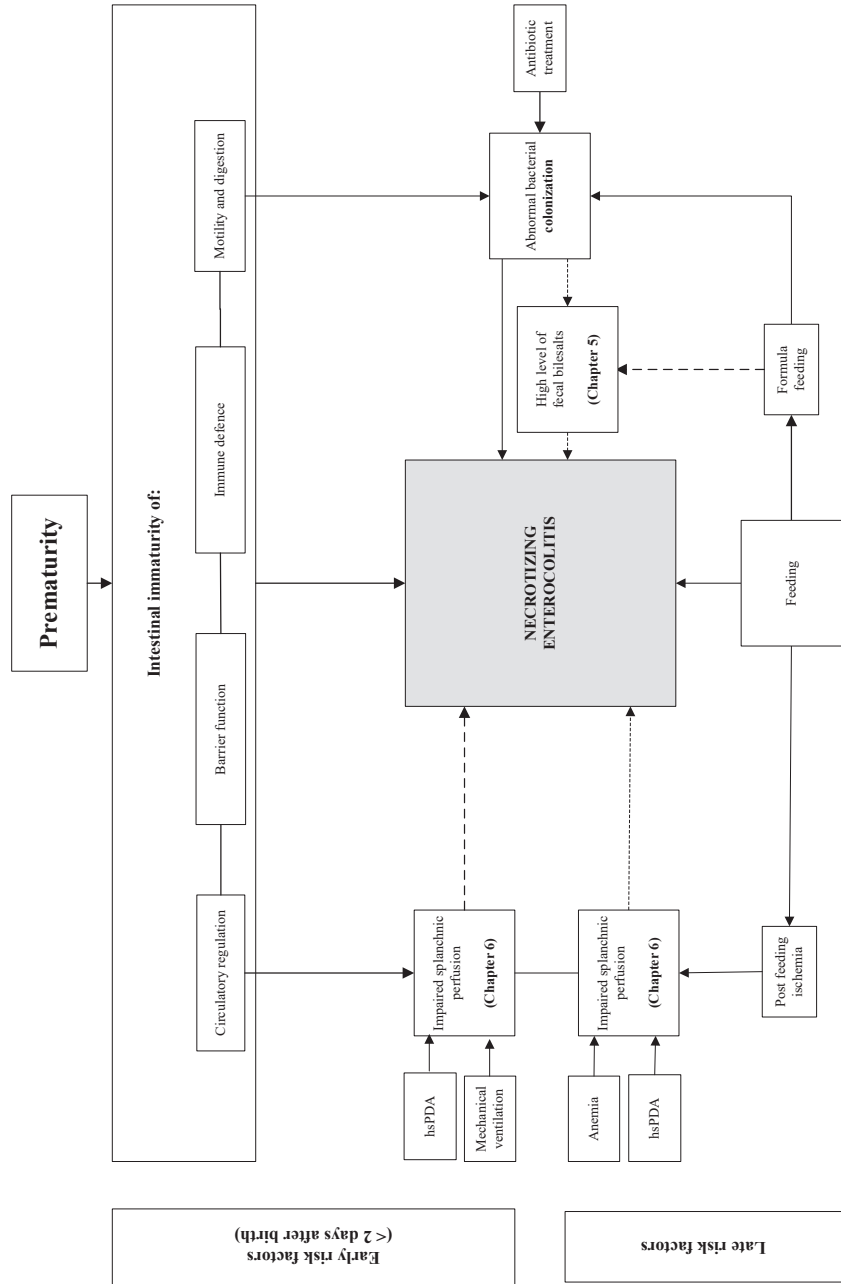


Figure 1. Schematic overview of the pathophysiology of necrotizing enterocolitis.

As decreased blood flow to the cerebral tissue usually occurs when compensatory flow redistribution, e.g. reduction of splanchnic circulation, has failed,³⁸ we speculate that the splanchnic perfusion also has been affected. The small sample size might have led to the absence of significant differences in intestinal oxygenation values between infants who developed necrotizing enterocolitis and controls. In line with previous studies we speculate that impaired intestinal circulation that occurs early after birth can predispose an infant to the development of necrotizing enterocolitis later in life.^{33,36,37} The direct effect of this impaired circulation on intestinal integrity seems, however, relatively mild since enterocyte damage is not yet detectable in the first week of life. We speculate, therefore, that a combination of several early and late postnatal factors eventually contribute to the development of necrotizing enterocolitis and related enterocyte damage (Figure 1).

Beside risk factors early after birth, several factors during the first week(s) of life might be related to the development of necrotizing enterocolitis later on. Numerous late factors might negatively influence the splanchnic perfusion, including anemia,^{39,40} relative ischemia during or after enteral feeding,^{41–43} or the presence of a hemodynamically significant patent ductus arteriosus (hsPDA).⁴⁴ All of these factors have been suggested previously to increase the risk of the development of necrotizing enterocolitis. Two days prior to the onset of necrotizing enterocolitis (at median postnatal day eight), we observed statistically significant higher values of intestinal FTOE in infants who developed necrotizing enterocolitis compared with controls (*Chapter 6*). This finding suggests that impaired splanchnic perfusion is present before the first clinical symptoms of necrotizing enterocolitis occur. The impaired circulation on intestinal integrity does, however, not seem to induce direct damage of the intestinal cells, as we found no differences in iFABP/cr_u ratios between subjects that developed necrotizing enterocolitis and controls in the week prior to the development of necrotizing enterocolitis (*Chapter 3*).

After the clinical suspicion of necrotizing enterocolitis had arisen, we clearly observed higher levels of iFABP/cr_u in necrotizing enterocolitis subjects compared to controls (*Chapter 3*). This is in line with previous data, also from our institution.⁴⁵ We therefore concluded that necrotizing enterocolitis is a disease in which intestinal damage and clinical signs go together. In the studies described in this thesis, we did not measure regional tissue oxygenation after the clinical suspicion of necrotizing enterocolitis. Data from another prospective trial in our center regarding the course of necrotizing enterocolitis (No-NEC-trial), however, showed a strong correlation between cerebral and splanchnic FTOE values with plasma iFABP levels during the first 16 hours after the onset of necrotizing enterocolitis.⁴⁶ We speculate, then, that the elevated iFABP/cr_u ratios we found after the clinical suspicion of necrotizing enterocolitis also have been accompanied by higher cerebral and splanchnic FTOE values. Nevertheless, it would be interesting to investigate whether this speculation

or hypothesis is true. If so, the combination of cerebral and splanchnic FTOE values with iFABP levels in urine might aid in the *noninvasive* prediction of the *progression* of necrotizing enterocolitis.

An obvious risk factor for necrotizing enterocolitis that is directly related to prematurity is the immaturity of the immune defense together with an excessive inflammatory response.³² It is unknown whether the onset of necrotizing enterocolitis is preceded by a low-grade inflammatory process that does not yet induce clinical signs. This inflammatory process might be accompanied by subclinical enterocyte damage. In the week prior to the onset of necrotizing enterocolitis, we found no differences in fecal calprotectin levels between infants who went on to develop necrotizing enterocolitis and controls (*Chapter 4*). Neither did we observe differences in iFABP/cr_u ratios between the two groups (*Chapter 3*). These findings suggest that there is no subclinical inflammatory process and accompanying enterocyte damage prior to the onset of necrotizing enterocolitis. We did find, however, high concentrations and wide interindividual variation in calprotectin levels in our study population when compared to calprotectin concentrations in children and adults. These high concentrations may reflect increased trans-epithelial migration of neutrophils into the intestinal lumen as a result of higher intestinal permeability during the neonatal period.^{22,47,48} Even so, this higher permeability does not seem to be related to the development of necrotizing enterocolitis later on, as concentrations of calprotectin were comparable in infants who did not develop the disease.

Another risk factor of the development of necrotizing enterocolitis might be the level of fecal bile salts. Intra-luminal accumulation of fecal bile salt levels can result in intestinal epithelial destruction. This epithelial damage is similar to histopathological findings in necrotizing enterocolitis.³⁵ In the first feces sample after birth i.e. at the 3rd postnatal day, we found no differences in both composition and concentration of fecal bile salt levels between infants who went on to develop necrotizing enterocolitis and infants who did not (controls). We observed, however, a slower decrease of total unconjugated fecal bile salt concentrations in subjects that developed necrotizing enterocolitis between birth and the onset of the disease when compared to controls. In the week prior to the development of necrotizing enterocolitis, total unconjugated bile salts were higher in infants developing necrotizing enterocolitis compared with levels measured in controls.

In line with previous studies we speculate that this transient failure to decrease bile salts coincides with an increased level of intra-enteric bile salts of the preterm intestine, predisposing an infant to the development of necrotizing enterocolitis. This leaves the question: how can increased levels of fecal bile salts be related to increased levels of intra-enterocyte bile salts? One answer to this question is that intracellular intestinal bile salts have been shown to decrease ileal mucin production and alter the intestinal mucus layer which might compromise the intestinal

barriers function.⁴⁹ Another possibility is involvement of apical sodium dependent bile acid transporter (ASBT), a protein involved in the luminal uptake of bile salts into the enterocyte.^{29,50} Necrotizing enterocolitis was attenuated in ASBT knockout mice and in rats treated with an ASBT inhibitor.⁵⁰ In accordance with the postulated role of increased bile-salt uptake at the distal small intestine, ASBT expression was increased in intestinal samples of preterm infants with necrotizing enterocolitis, compared with ileal surgical samples from preterm infants with other diseases.⁵⁰ Interestingly, it has recently been suggested that the expression of ASBT is influenced by the intestinal microbiota.²⁹ Under physiological circumstances, the microbiota causes a suppression of ASBT expression, limiting the expression of this transporter to the terminal ileum. An alteration of the intestinal microbiota, as a result of, for instance, antibiotic treatment or formula feeding, might lead to a change in ASBT expression and hence to increased levels of intra-enterocyte bile salts. Whether the course of fecal bile salts in preterm infants is influenced by antibiotic treatment or type of feeding is unknown, just as it is unclear whether this course of bile salts is related to the microbiota, iFABP levels or circulatory measurement.

In conclusion, the results of this thesis confirm the prevailing idea that preterm necrotizing enterocolitis is a multifactorial disease. Those infants who develop necrotizing enterocolitis do so because they have acquired multiple distinct risk factors. The direct effect of these factors on intestinal integrity seems relatively mild since enterocyte damage is not detectable prior to the first clinical signs of the disease. The first 'hit' (e.g. a reduction of intestinal circulation) might occur even before birth, or during the first days after birth and predispose an infant to the development of necrotizing enterocolitis later in life. When this first hit is followed by multiple distinct factors that have additive risk, such as anemia and the presence of an hsPDA that impair the intestinal circulation or antibiotic treatment and formula feeding that might affect the levels of bile salts, the preterm infant might go on to develop necrotizing enterocolitis. The coincidence of risk factors seems to be an acute event, in which damage of the intestinal cells rapidly leads to clinical signs.

Future perspectives

The markers we described in this thesis were all investigated separately for their role in the identification of infants who are most likely to develop necrotizing enterocolitis. It would be very interesting and of added value to determine the potential predictive power of combining these different markers.

We mentioned that measuring cerebral and splanchnic oxygenations might be useful in the early identification of preterm infants who will develop necrotizing enterocolitis. The results were, however, found in a relatively small number of infants and the clinical relevance of these values needs further determination. The

usefulness of monitoring cerebral and splanchnic oxygenation values to predict the onset of necrotizing enterocolitis needs to be investigated further in a larger, preferably multi-center, trial. Until data of such a trial becomes available, we suggest that infants with low cerebral oxygenation saturation within the first two days after birth should be monitored more intensively in order to notify the first clinical signs of necrotizing enterocolitis as early as possible.

Although no specific change in the intestinal microbiota has been implicated in necrotizing enterocolitis so far, disturbance of the colonization patterns in the developing intestine is thought to play a role in the pathophysiology of the disease.^{11,51} Recent advances in 16S rRNA based sequencing technologies allow for detailed analysis of the bacterial composition of feces, including the accurate measurement of unculturable bacteria.⁵²⁻⁵⁴ Further studies are needed to determine the composition of the intestinal microbiota in preterm neonates and its relation to the development of necrotizing enterocolitis later on. Early microbial signatures in both feces and urine should also be studied, as they might provide highly predictive biomarkers of necrotizing enterocolitis.⁵¹

Both an altered microbiota and formula feeding are thought to influence the levels of fecal bile salts.^{28,29} As we found higher levels of fecal bile salts prior to the development of necrotizing enterocolitis, it would be very interesting to investigate the precise effect of the microbiota, antibiotic treatment and type of feeding on levels of fecal bile salts in preterm infants, and determine their role in the pathophysiology of necrotizing enterocolitis.

Studies on non-invasive markers in the prediction of necrotizing enterocolitis remain essential in the near future. These studies should, however, go together with studies regarding the unraveling of the pathophysiology of necrotizing enterocolitis. The more we understand the pathophysiology of this devastating disease, the better we will be able to strike out preventative strategies, which may subsequently improve neonatal outcome. Prevention of this highly damaging disease should be considered the common goal for all researchers in the field of necrotizing enterocolitis.

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